09-22-00

.7

jc490 U.S. PTO 09/21/00

Please type a plus sign (+) inside this box → [+]

PTO/SB/05 (1/98)
Approved for use through 09/30/2000. OMB 0651-0032
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	4205.1US
First Inventor or Application Identifier	Delphine Gabrielle Josette Rea et al.
Title	See 1 in Addendum
Express Mail Label No.	EL700256994US

jc905 U.S. PTO 09/21/00

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231

1. [X] * Fee Transmittal Form *(e.g., PTO/SB/17)* *(Submit an original, and a duplicate for fee processing)*
2. [X] Specification [Total Pages 27] *(preferred arrangement set forth below)*
   - Descriptive title of the Invention
   - Cross References to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to Microfiche Appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
3. [X] Drawing(s) *(35 U.S.C. 113)* [Total Sheets 6]
4. Oath or Declaration [Total Pages 2]
   a. [X] Newly executed (original or copy)
   b. [ ] Copy from a prior application (37 C.F.R. § 1.63(d)) *(for continuation/divisional with Box 17 completed)* ***[Note Box 5 below]***
      i. [ ] DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. §§ 1.63(d)(2) and 1.33(b).
5. [ ] Incorporation By Reference *(useable if Box 4b is checked)* The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered to be part of the disclosure of the accompanying application and is hereby incorporated by reference therein.
6. [ ] Microfiche Computer Program *(Appendix)*
7. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. [ ] Computer Readable Copy
   b. [ ] Paper Copy (identical to computer copy)
   c. [ ] Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

8. [ ] Assignment Papers (cover sheet & document(s))
9. [ ] 37 C.F.R.§3.73(b) Statement *(when there is an assignee)* [ ] Power of Attorney
10. [ ] English Translation Document *(if applicable)*
11. [ ] Information Disclosure Statement (IDS)/PTO-1449 [ ] Copies of IDS Citations
12. [X] Preliminary Amendment
13. [X] Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
14. [X] * Small Entity Statement(s) *(PTO/SB/09-12)* [ ] Statement filed in prior application, Status still proper and desired
15. [ ] Certified Copy of Priority Document(s) *(if foreign priority is claimed)*
16. [ ] Other: .....

* *A new statement is required to be entitled to pay small entity fees, except where one has been filed in a prior application and is being relied upon.*

17. **If a CONTINUING APPLICATION,** *check appropriate box, and supply the requisite information below and in a preliminary amendment:*
    [ ] Continuation [ ] Divisional [ ] Continuation-in-part (CIP) of prior application No: _____/_____
    Prior application information: Examiner _____ *Group / Art Unit:* _____

18. CORRESPONDENCE ADDRESS

[X] *Customer Number or Bar Code Label* *(Insert Customer No. or Attach bar code label here)* or [ ] *Correspondence address below*

Name	Allen C. Turner Trask Britt				
Address	P.O. Box 2550				
City	Salt Lake City	State	Utah	Zip Code	84110
Country	U.S.A.	Telephone	(801) 532-1922	Fax	(801) 531-9168

Name (Print/Type)	Allen C. Turner	Registration No. (Attorney/Agent)	33,041
Signature		Date	09/21/00

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231

Addendum

1. DENDRITIC CELL ACTIVATED IN THE PRESENCE OF GLUCOCORTICOID HORMONES ARE CAPABLE OF SUPPRESSING ANTIGEN-SPECIFIC T CELL RESPONSES

PTO/SB/17 (12/99)
Approved for use through 09/30/2000. OMB 0651-0032
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

FEE TRANSMITTAL for FY 2000

Patent fees are subject to annual revision.
Small Entity payments must be supported by a small entity statement, otherwise large entity fees must be paid. See Forms PTO/SB/09-12. See 37 C.F.R. §§ 1.27 and 1.28.

TOTAL AMOUNT OF PAYMENT ($) 546.00

Complete if Known	
Application Number	
Filing Date	September 21, 2000
First Named Inventor	Rea et al.
Examiner Name	
Group / Art Unit	
Attorney Docket No.	4205.1US

METHOD OF PAYMENT (check one)

1. [X] The Commissioner is hereby authorized to charge indicated fees and credit any overpayments to:

Deposit Account Number: 20-1469

Deposit Account Name: Trask Britt

[X] Charge Any Additional Fee Required Under 37 CFR §§ 1.16 and 1.17

2. [X] **Payment Enclosed:**
[X] Check [ ] Money Order [ ] Other

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
101	690	201	345	Utility filing fee	345
106	310	206	155	Design filing fee	0
107	480	207	240	Plant filing fee	0
108	690	208	345	Reissue filing fee	0
114	150	214	75	Provisional filing fee	0

SUBTOTAL (1) ($) 345.00

2. EXTRA CLAIM FEES

		Extra Claims		Fee from below		Fee Paid
Total Claims	25 -20** =	5	X	9	=	45
Independent Claims	7 - 3** =	4	X	39	=	156
Multiple Dependent				0	=	0

**or number previously paid, if greater; For Reissues, see below

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
103	18	203	9	Claims in excess of 20
102	78	202	39	Independent claims in excess of 3
104	260	204	130	Multiple dependent claim, if not paid
109	78	209	39	** Reissue independent claims over original patent
110	18	210	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 201.00

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
105	130	205	65	Surcharge - late filing fee or oath	
127	50	227	25	Surcharge - late provisional filing fee or cover sheet.	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	380	216	190	Extension for reply within second month	
117	870	217	435	Extension for reply within third month	
118	1,360	218	680	Extension for reply within fourth month	
128	1,850	228	925	Extension for reply within fifth month	
119	300	219	150	Notice of Appeal	
120	300	220	150	Filing a brief in support of an appeal	
121	260	221	130	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,210	241	605	Petition to revive - unintentional	
142	1,210	242	605	Utility issue fee (or reissue)	
143	430	243	215	Design issue fee	
144	580	244	290	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Petitions related to provisional applications	
126	240	126	240	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	690	246	345	Filing a submission after final rejection (37 CFR § 1.129(a))	
149	690	249	345	For each additional invention to be examined (37 CFR § 1.129(b))	

Other fee (specify) _____

Other fee (specify) _____

* Reduced by Basic Filing Fee Paid

SUBTOTAL (3) ($)

SUBMITTED BY				Complete (if applicable)	
Name (Print/Type)	Allen C. Turner	Registration No. (Attorney/Agent)	33,041	Telephone	(801) 532-1922
Signature				Date	09/21/2000

WARNING:

Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

Burden Hour Statement. This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

Applicant or Patentee: Delphine Gabrielle Josette Rea; Cornelis Johannes Maria Melief; Rienk Offringa Attorney Docket No. 4205.1US
Serial No.: _____ Filed: _____
For: DENDRITIC CELL ACTIVATED IN THE PRESENCE OF GLUCOCORTICOID HORMONES ARE CAPABLE OF SUPPRESSING ANTIGEN-SPECIFIC T CELL RESPONSES

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 C.F.R. §§ 1.9(f) and 1.27 (d)) - NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION Leids Universitair Medisch Centrum
ADDRESS OF ORGANIZATION Albinusdreef 2, 2333 ZA Leiden, The Netherlands

TYPE OF ORGANIZATION

- ☒ University or other institution of higher education
- ☐ Tax exempt under Internal Revenue Service Code (26 U.S.C. § 501(a) and 501(c)(3))
- ☐ Nonprofit scientific or educational under statute of state of The United States of America
  (Name of state: _____)
  (Citation of statute: _____)
- ☐ Would qualify as tax exempt under Internal Revenue Service Codes (26 U.S.C. § 501(a) and 501(c) if located in The United States of America
- ☐ Would qualify as nonprofit scientific or educational under statute of state of The United States of America if located in The United States of America
  (Name of state: _____)
  (Citation of statute: _____)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 C.F.R. § 1.9(e) for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code with regard to the invention entitled DENDRITIC CELL ACTIVATED IN THE PRESENCE OF GLUCOCORTICOID HORMONES ARE CAPABLE OF SUPPRESSING ANTIGEN-SPECIFIC T CELL RESPONSES by inventor(s) Rea et al. described in

- ☒ the specification filed herewith.
- ☐ application serial no. _____, filed _____.
- ☐ patent no. _____, issued _____.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above-identified invention.

If the rights held by the above-identified nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 C.F.R. § 1.9(c) if that person made the invention or by any concern which would not qualify as a small business concern under 37 C.F.R. § 1.9(d) or a nonprofit organization under 37 C.F.R. § 1.9(e).

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 C.F.R. § 1.27)

NAME: _____
ADDRESS: _____
☐Individual ☐Small Business Concern ☐Nonprofit Organization

NAME: _____
ADDRESS: _____
☐Individual ☐Small Business Concern ☐Nonprofit Organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate (37 C.F.R. § 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: Carlo H.G.
TITLE IN ORGANIZATION: LUMC
ADDRESS OF PERSON SIGNING: Albinusdreef 2 Leiden
SIGNATURE: _____ DATE: 13/07/00

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Rea et al.

Serial No.: To be assigned

Filed: September 21, 2000

For: DENDRITIC CELLS ACTIVATED IN THE PRESENCE OF GLUCOCORTICOID HORMONES ARE CAPABLE OF SUPPRESSING ANTIGEN-SPECIFIC T CELL RESPONSES

Examiner: To be assigned

Group Art Unit: To be assigned

Attorney Docket No.: 4205.1US

NOTICE OF EXPRESS MAILING

Express Mail Mailing Label Number: EL700256994US

Date of Deposit with USPS: September 21, 2000

Person making Deposit: Amanda Trulson

Preliminary Amendment

Commissioner for Patents
Washington, D.C. 20231

Sir:

Before calculating the filing fee, please amend the referenced application as follows:

IN THE TITLE:

Please replace "Cell" with –Cells–.

IN THE SPECIFICATION:

Page 2, line 2, please replace "(GC)" with –(the abbreviation "GC" is used herein for the terms "glucocorticoids" and "glucocorticoid")–.

Page 3, line 7, please insert the heading –Summary of The Invention– centered on the line.

Page 4, line 21, please insert enclosed substitute pages numbered 4a-4c.

Page 4, line 22, please insert the heading –Detailed Description of The Preferred Embodiments– centered on the line.

Page 4, line 31, please insert a comma after "aspect";

Page 4, line 31, please insert –the– before "invention".

Page 5, line 11, please insert a comma after "Preferably".

Page 5, line 15, please replace "at risk" with –"at risk", it–.

Page 5, line 18, please insert –be– between "to" and "at".

Page 5, line 28, please insert a comma after "aspect".

Page 5, line 35, please insert a comma after "embodiment".

Page 6, line 11, please insert a comma after "invention".

Page 6, line 20, please insert a comma after "aspect".

Page 6, line 33, please insert a comma after "embodiment".

Page 7, line 1, please insert a comma after "aspect".

Page 7, line 15, please insert a comma after "aspect".

Page 7, line 33, please replace "use" with –used–.

Pages 15 through 17, please delete all text included on these pages.

Page 26, before the text entitled "**ABSTRACT**", please delete "Title: Dendritic cell activated in the presence of glucocorticoid hormones are capable of suppressing antigen-specific T cell responses."

IN THE CLAIMS:

1. (Amended) A method for preparing a pharmaceutical composition for reducing an unwanted T-cell response in a host, comprising:

culturing peripheral blood monocytes from said host to differentiate into dendritic cells[,];

activating said dendritic cells in the presence of a glucocorticoid hormone; and

loading said dendritic cells with an antigen against which said T-cell response is to be reduced.

4. (Amended) A method for reducing an unwanted T-cell response in a host, comprising:

culturing peripheral blood monocytes from said host to differentiate into dendritic cells[,];

activating said dendritic cells in the presence of a glucocorticoid hormone [and];

loading said dendritic cells with an antigen against which said T-cell response is to be reduced;

forming a composition comprising said dendritic cells loaded with an antigen against which said T-cell response is to be reduced; and

administering said composition to said host.

5. (Amended) [A] The method according to claim 1, [3 or 4 whereby said activation is done through] wherein activating said dendritic cells in the presence of a glucocorticoid hormone comprises activating said dendritic cells through a CD40 receptor.

6. (Amended) [A] The method according to claim 5 [whereby said activation], wherein activating said dendritic cells through a CD40 receptor involves incubation of the dendritic cells with [either] a substance selected from a group consisting of a CD8-40L fusion protein, a trimeric form of CD40L consisting of CD40L molecules to which a modified leucine zipper has been attached, anti-CD40 antibodies, [or] and cells that express CD40L.

7. (Amended) [A] The method according to claim 5 [whereby said activation], wherein activating said dendritic cells through a CD40 receptor involves incubation of the dendritic cells with a substance selected from a group consisting of lipopolysaccharide (LPS) [or] and polyI/C.

8. (Amended) [A] The method according to claim 1, [3 -7 whereby] further comprising infecting said dendritic cells [are infected] with one or more recombinant viruses encoding [the antigen(s)] at least one antigen of interest before activating said dendritic cells in the presence of a glucocorticoid hormone.

9. (Amended) [A] The method according to claim 1, [3-8 whereby] further comprising incubating said dendritic cells [are incubated] with [one or more recombinant proteins or large (> 20 amino acids) synthetic] at least one peptide representing [the antigen(s)] at least one antigen of interest before activating said dendritic cells in the presence of a glucocorticoid hormone.

10. (Amended) [A] The method according to claim 1, [3-9 whereby] further comprising incubating said dendritic cells [are incubated] with cells [or cell homogenate] containing [the antigen(s)] at least one antigen of interest before activating said dendritic cells in the presence of a glucocorticoid hormone.

11. (Amended) [A] The method according to claim 1, [3-10 whereby said dendritic cells are loaded] wherein loading said dendritic cells with an antigen against which said T-cell response is to be reduced comprises loading said dendritic cells with at least one synthetic [peptide(s)] peptide representing [the antigen(s)] at least one antigen of interest after activating said dendritic cells in the presence of a glucocorticoid hormone.

12. (Amended) [A] The method according to claim 1, [3-11 whereby said dendritic cells, after activation] wherein activating said dendritic cells in the presence of a glucocorticoid

hormone[,] comprises activating said dendritic cells such that said dendritic cells secrete interleukin-10.

13. (Amended) A method for obtaining a dendritic cell capable of tolerising a T-cell for an antigen, comprising:

providing said dendritic cell with a glucocorticoid hormone[,];

activating said dendritic cell; and

providing said dendritic cell with said antigen.

14. (Amended) [A] The method according to [anyone of claims 1, 3-13,] claim 13, wherein providing said dendritic cell with a glucocorticoid hormone comprises providing said dendritic cell [and/or a precursor thereof is provided] with said glucocorticoid hormone in vitro.

15. (Amended) [A] The method according to [anyone of claim 1, 3-14,] claim 1, wherein said T-cell is a T-helper cell.

16. (Amended) An isolated dendritic cell [prepared according to anyone of claims 1, 3-15] capable of functionally modifying [an antigen-specific] a T-cell [with respect to the response to said antigen] specific to an antigen such that the response of said T-cell to said antigen is altered.

17. (Amended) A method for functionally modifying [an antigen-specific] a T-cell specific to an antigen, comprising:

providing [an] a dendritic cell [according to claim 16 with said antigen]capable of functionally modifying said T-cell such that the response of T-cell to said antigen is altered; and

co-cultivating said T-cell and said dendritic cell.

18. (Amended) [A] The method according to claim 17, wherein [said] co-cultivating said T-cell and said dendritic cell comprises co-cultivating said T-cell and said dendritic cell [is

performed] in vitro.

19. (Amended) [A] The method according to claim 17 [or claim 18], further comprising multiplying said functionally modified T-cell.

20. (Amended) An isolated functionally modified T-cell [obtainable by a method according to anyone of claims 17-19]produced by the process of claim 17 that is capable, upon administration to a host, of reducing an unwanted immune response.

22. (Amended) A pharmaceutical composition comprising [an dendritic cell according to claim 16 and/or a functionally modified T-cell according to claim 20] a cell selected from a group consisting of a dendritic cell capable of functionally modifying a T-cell specific to an antigen such that the response of said T-cell to said antigen is altered and a functionally modified T-cell capable of reducing an unwanted immune response upon administration to a host.

24. (Amended) A method for the treatment of an individual suffering from or at risk of suffering from a disease associated with at least part of the immune system of said individual, the method comprising:

providing said individual with [an] a cell selected from a group consisting of a dendritic cell [according to claim 16 and/or] capable of functionally modifying a T-cell specific to an antigen such that the response of said T-cell to said antigen is altered and a functionally modified T-cell [according to claim 20] capable of reducing an unwanted immune response upon administration to a host.

25. (Amended) [A] The method according to claim 24, wherein [said dendritic cell and or said T-cell] providing said individual with a cell comprises providing a cell that is derived from an HLA-matched donor.

26. (Amended) [A] The method according to claim 24, wherein [said dendritic cell

and or said T-cell is] providing said individual with a cell comprises providing a cell derived from said individual.

27. (Amended) [Use of an dendritic cell according to claim 16 in a treatment for] A method of treating an individual suffering from a disease selected from a group consisting of an auto-immune disease, an allergy, a graft versus host disease, [and/or] and a host versus graft disease, comprising:

providing an isolated dendritic cell capable of functionally modifying a T-cell specific to an antigen such that the response of said T-cell to said antigen is altered; and

introducing said isolated dendritic cell into said individual.

Please add the following new claims:

28. The method according to claim 1, further comprising incubating said dendritic cells with cell homogenate containing at least one antigen of interest before activating said dendritic cells in the presence of a glucocorticoid hormone.

29. The method according to claim 13, wherein providing said dendritic cell with a glucocorticoid hormone comprises providing a precursor of said dendritic cell with said glucocorticoid hormone in vitro.

Please cancel claims 2, 3, 21 and 23 without prejudice or disclaimer.

Remarks

The application is to be amended as previously set forth. The changes are generally made to correct minor typographical and format errors and to more appropriately claim the invention in view of United States practice. As indicated, substitute pages 1a, 1b, and 4a-4c are enclosed herewith in order to simplify amendment of the application. It is respectfully submitted that no new matter has been added.

Conclusion

In the event questions remain after consideration of these amendments, the Office is kindly requested to contact applicant's attorney at the number given below.

Respectfully submitted,

Allen C. Turner
Registration No. 33,041
Attorney for Applicant
TRASK BRITT, PC
P. O. Box 2550
Salt Lake City, Utah 84110-2550
Telephone: (801) 532-1922

Date: September 21, 2000

Enclosures: Substitute pages 4a-4c

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 Pretreatment with DEX inhibits the phenotypic changes induced by CD40 ligation.
Seven days immature DC were cultured for 24h in the absence or the presence of 10^{-6}M DEX and activated via CD40 with the CD8-CD40L fusion protein for 48h. The comparison with immature DC maintained in medium alone is shown. Empty histograms show the background staining with isotype controls MoAb and solid histograms represent specific staining of the indicated cell surface markers. Specific mean fluorescence intensities are indicated. Mean fluorescence intensities of isotype controls were between 3 and 4. Data are representative of 4 independent experiments.

Fig. 2 DC triggered through CD40 maintain an activated phenotype upon a subsequent DEX exposure.
Immature DC were activated with the CD8-CD40L fusion protein. DEX (10^{-6}M) or medium control were added 48h later and cells were analyzed after 2 additional days of culture. The comparison with immature DC maintained in medium alone is shown. Empty histograms show the background staining with isotype controls MoAb and solid histograms represent specific staining of the indicted cell surface markers. Specific mean fluorescence intensities are indicated. Mean fluorescence intensities of isotype controls were between 3 and 5. Data are representative of 2 independent experiments.

Fig. 3 Pretreatment with DEX does not affect the regulation of DC antigen uptake machinery.
Immature DC were incubated in the absence or the presence of

10^{-6}M DEX for 24h and further activated or not via CD40 with the CD8-CD40L fusion protein for 48h. Cells were pulsed for 1h with medium containing either 1mg/ml FITC-BSA or 1mg/ml FITC-mannosylated BSA. Empty histograms show the background autofluorescence, Grey-filled histograms show the background uptake at 0°C. and black-filled histograms show the specific uptake at 37°C. Data are representative of 3 independent experiments.

Fig. 4 Pretreatment with DEX alters the cytokine secretion profile of CD40-triggered DC.
DEX-exposed or control immature DC were left in culture without further treatment or stimulated with the CD8-CD40L fusion protein. Culture supernatants were harvested 48h later and IL-10, IL-12p40 and IL-12p70 secretion were analyzed by specific ELISA. Data are representative from 6 independent experiments.

Fig. 5 Pretreatment with DEX impairs the T cell stimulatory capacities of DC activated via CD40 and leads to a state of hyporesponsiveness of Th1 cells.
Allogeneic MLR: non adherent allogeneic PBMC were cultured with different numbers of CD40-triggered DC, DEX-treated CD40-triggered DC or immature DC. The proliferative response was measured on day 5.
Th1 stimulation assays: Hsp65-specific T cells were cultured with different numbers of HLA-DR matched CD40-triggered DC or with DEX-treated CD40-triggered DC pulsed with the hsp65 protein or with the specific p3-13 peptide epitope. The proliferative response and the T cell dependent IFN-g production were analyzed on day 3. Data are representative of 4 independent experiments.

Fig. 6 DEX-treated DC triggered through CD40 induce a state of hyporesponsiveness in Th1 cells. Hsp65-specific T cells precultured with CD40-triggered DC or with DEX-treated CD40-triggered DC pulsed with the p3-13 peptide epitope were harvested after 48h, allowed to rest in the presence of 5U/ml IL2 for 3 days, and restimulated with p3-13-pulsed DC. The proliferative response and IFN-g production were measured on day 3. Similar results were obtained in 2 independent experiments.

PATENT
Attorney Docket 4205.1US

NOTICE OF EXPRESS MAILING

Express Mail Mailing Label Number: EL700256994US

Date of Deposit with USPS: September 21, 2000

Person making Deposit: Amanda Trulson

APPLICATION FOR LETTERS PATENT

for

DENDRITIC CELL ACTIVATED IN THE PRESENCE OF GLUCOCORTICOID HORMONES ARE CAPABLE OF SUPPRESSING ANTIGEN-SPECIFIC T CELL RESPONSES

Inventors:

Delphine Gabrielle Josette Rea
Cornelis Johannes Maria Melief
Rienk Offringa

Attorney:
Allen C. Turner
Registration Number: 33,041
Trask Britt
P.O. Box 2550
Salt Lake City, Utah 84111
(801) 532-1922

DENDRITIC CELL ACTIVATED IN THE PRESENCE OF GLUCOCORTICOID HORMONES ARE CAPABLE OF SUPPRESSING ANTIGEN-SPECIFIC T CELL RESPONSES

Reference to Related Application: This application claims priority from Provisional Application Serial No. 60/157,442, filed October 4, 1999.

Field: The invention relates to the field of medicine. More in particular the invention relates to the field of immunotherapy.

Background: The remarkable immunostimulatory properties of dendritic cells ("DC") reside in their ability to transport antigens from peripheral tissues to lymphoid organs where they present these antigens to T cells in an optimal costimulatory context. (1). To achieve this complex sequence of events, DC exist in different functional stages. Immature DC behave as sentinels in peripheral tissues where they efficiently capture intigens. Upon pathogen invasion, induction of protective T cell responses requires the activation of immature DC into mature immunostimulatory cells. DC activation is triggered in inflamed tissues by cytokines such as IL-1 and TNF-a and by bacterial components such as LPS (2, 3). Activated DC migrate to T cell areas in the lymph nodes while upregulating their costimulatory capacities and optimizing their antigen presenting functions. Upon interaction with antigen-specific T cells, DC activation is further completed through engagement of the receptor-ligand (l) pair CD40-CD40L, leading to the production of IL-12 (4, 5, 6), a key cytokine for T helper (Th) type 1 and cytotoxic T lymphocyte (CTL) priming (7).

APC activation through CD40-CD40L interactions represents an important immunoregulatory step for the establishment of protective T cell immunity against pathogens and tumors (8, 9, 10). This process also plays a key role in the onset of destructive T cell-mediated disorders such as auto-immune diseases, allograft rejection and graft versus host disease (11, 12, 13). The current treatment of these

disorders largely relies on the administration of glucocorticoids (GC), which exert potent anti-inflammatory and immunosuppressive effects. Because GC negatively interfere with many aspects of T cell activation such as IL-2-driven proliferation and inflammatory cytokine production (reviewed in 14), activated T cells have long been considered as the main targets for GC action. Several lines of evidence now suggest a role for DC in GC-induced immune suppression. Moser et al (15) found that GC prevented the spontaneous activation of murine DC thereby decreasing their T cell stimulatory potential. Kitajima et al (16) showed that GC could hamper the T cell-mediated activation of a murine DC line. Viera et al reported that human DC exposed to GC were poor producers of IL-12 upon LPS stimulation (17). These findings only concern loss of typical DC features and therefore favor a simple inhibitory role of GC on DC activation. A more complex immunoregulatory action on the DC system has not been considered.

The present invention resulted from a detailed analysis of the impact of GC on the CD40-mediated activation of monocyte-derived DC. These DC develop after culture with GM-CSF and IL-4 (2, 18) or after transmigration through endothelial cells (19) and are known to mature into the most potent human Th1-type-inducing APC upon CD40 ligation (5, 20). Moreover, these APC can easily be generated in large numbers and are thereby the cells of choice for DC-based modulation of T cell immunity (21, 22). In contrast to previous studies, the present invention shows that GC such as dexamethasone (DEX) do not merely prohibit DC activation, but that it converts CD40 ligation on human monocyte-derived DC is transformed into an alternative activation pathway. DEX profoundly affect the CD40-dependent maturation of human monocyte-derived DC, not only by preventing the upregulation of costimulatory, adhesion and MHC surface molecules, but also by causing these cells to secrete the anti-inflammatory mediator IL-10 instead of the Th1 stimulatory cytokine IL-12. In agreement with

these phenotypic and functional changes, DC triggered through CD40 in the presence of DEX are poor stimulators of Th1-type responses. Most importantly, the present invention shows that such DC are able to induce a state of hyporesponsiveness in Th1 cells, indicating that these cells are capable of active suppression of Th1-type immunity.

As already mentioned above, the impact of GC on DC has been the subject of several previous studies by others. However, in contrast with the present invention, these studies only highlighted inhibitory effects of GC on the DC system. DEX was found to block the upregulation of CD80, CD86 and MHC class II molecules upon activation of murine spleen DC (15, 16), whereas very recently DEX was demonstrated to also prevent the differentiation of DC from monocyte precursors (28). In these studies, the inability of DC to acquire high expression of costimulatory and MHC molecules was accompanied with a decrease in their T cell stimulatory potential, but the effect of GC on IL-12 production was not investigated. On the other hand, Viera et al found that the effect of GC on LPS-induced DC activation consisted in a 4-fold reduction of IL-12p70 synthesis (17). This partial effect on IL-12 secretion contrasts with the complete suppression of IL-12p70 production which is subject of the present invention, and can be explained by the fact that their GC-treated immature DC were extensively washed prior to LPS stimulation. We indeed found that upon removal of GC, the effects of these drugs on immature DC were rapidly reversible. The continuous presence of GC during CD40 triggering of DC was clearly preferred in order to stably and completely modulate DC activation (data not shown). Taken together, previous findings indicated that the impact of GC on the DC system should be merely interpreted as an inhibitory event. Importantly, the present invention clearly demonstrates that GC such as DEX do not simply suppress DC activation but rather redirect this process towards a distinct functional program.

DC activation through engagement of CD40-CD40L is a key stimulatory event for the generation of effective Th1 and CD4-dependent CTL responses in vivo (10, 36, 37, 38). This pathway however is also involved in the development of unwanted T cell responses leading to autoimmune disease or organ-transplant rejection (11, 12, 13). Until now, treatment of patients suffering from such disorders largely relies on the systemic administration of GC hormones. This treatment does not only suppress pathogenic T cell responses but also induces a general state of immunosuppression and metabolic and endocrine side effects. The present invention demonstrates that activation of human monocyte-derived DC through CD40, in the presence of GC such as DEX, results in an IL-10-producing APC that is a poor stimulator for Th1-type responses and that can even confer hyporesponsiveness to Th1 cells. The present invention therefore indicates that such DC loaded with appropriate antigens can be exploited as a novel approach for specifically downregulating unwanted T cell responses in vivo.

The dentritic cells of the invention posses different capabilities than previously reported for dendritic cells. One can therefore consider these cells to be part of a class of cells distinct from the class formed by the "classical" dendritic cells. The dendritic cells of the invention can be used in a different way than the classical dendritic cells. The dendritic cells of the invention can for instance be used to suppress, at least in part, an undesired immune response in a host. In one aspect invention therefore provides a method for preparing a pharmaceutical composition for reducing an unwanted T cell response in a host, comprising culturing peripheral blood monocytes from said host to differentiate into dendritic cells, activating said dendritic cells in the presence of a glucocorticoid hormone and loading

said activated dendritic cells with an antigen against which said T cell response is to be reduced. An unwanted T cell response can be any type of T cell response. For instance, but not limited to, a T cell response associated with an auto-immune disease or a transplantation disease such as a graft versus host disease or a host versus graft disease. A pharmaceutical composition of the invention typically comprises a dendritic cell of the invention suspended in a liquid suitable for preserving the function of said dendritic cell in said liquid and/or suitable for administration to a host. A host preferably is a human. Preferably said host is at risk of developing or is suffering from an auto-immune disease or allergy. Preferably, said host suffers from or is at risk of suffering from host versus graft disease and/or graft versus host disease. With the term at risk is meant that one expects that said host may develop said disease, for instance but not limited to a host receiving a transplant. Such a host is considered to at risk of developing host versus graft disease. An antigen typically is a peptide capable of binding to a major histocompatibility complex I and/or II molecule. Such peptides are known in the art and a person skilled in the art is capable of determining whether a given peptide comprises an antigen or not. An antigen may be derived from a naturally occurring protein. An antigen may also be a synthetic peptide or equivalent thereof, preferably with an amino-acid sequence equivalent to a peptide derived from a protein.

In another aspect the invention provides a pharmaceutical composition for reducing an unwanted T cell response in a host, said composition being obtained by culturing peripheral blood monocytes from said host to differentiate into dendritic cells, activating said dendritic cells in the presence of a glucocorticoid hormone and loading said activated dendritic cells with an antigen against which said T cell response is to be reduced. In one embodiment a method is provided for reducing an unwanted T cell response in a

host, comprising administering a composition of the invention to said host.

The invention further provides method for reducing an unwanted T cell response in a host comprising culturing peripheral blood monocytes from said host to differentiate into dendritic cells, activating said dendritic cells and/or their precursors in the presence of a glucocorticoid hormone and loading said activated dendritic cells with an antigen against which said T cell response is to be reduced and administering said composition to said host.

In one embodiment of the invention said activation is done through a CD40 receptor. Activation of DC through triggering of the CD40 receptor can involve either incubation with a CD8-CD40L fusion protein, a trimeric from of CD40L consisting of CD40L-molecules to which a modified leucine zipper has been attached, anti-CD40 antibodies, or cells that express CD40L. Other signals that can be employed for the activation of DC as described in the present invention include lipopolysaccharide (LPS) and polyI/C.

In another aspect the invention provides a method for obtaining an dendritic cell capable of tolerising a T-cell for an antigen comprising providing said dendritic cell with a glucocorticoid hormone, activating said dendritic cell and providing said dendritic cell with said antigen. With the term tolerising is meant that said dendritic cell has an immunosuppressive effect on said T cell. A tolerised T cell will essentially not respond with cell division when exposed to a cell presenting an antigen said T cell would in the untolerised state respond to with cell division. A tolerised T cell will essentially not respond with killing a cell presenting an antigen said T cell would in the untolerised state respond to with cell kill.

In one embodiment said dendritic cell and/or a precursor thereof is provided with said glucocorticoid hormone <u>in vitro</u>. A T cell of the invention is preferably an antigen specific T cell, preferably a cytotoxic T cell or a Th cell.

In another aspect the invention provides an isolated dendritic cell capable of modifying the function of an antigen specific Th cell ,which would otherwise enhance a given immune response, resulting in a T cell that is capable of reducing this immune response. In one embodiment the invention provides a method for modifying an antigen specific T-cell comprising providing an dendritic cell according to the invention with said antigen and co-cultivating said T-cell and said dendritic cell. Preferably, said co-cultivating is performed in vitro. Said method may further comprise multiplying said functionally modified T-cell.

The invention also provides an isolated funtionally modified T-cell obtainable by a method according to the invention.

In another aspect the invention provides the use of a glucocorticoid hormone for obtaining an dendritic cell capable of functionally modifying a T-cell.

The invention also provides a pharmaceutical composition comprising an dendritic cell and/or a functionally modified T-cell according to the invention. The invention further provides the use of a dendritic cell and/or a functionally modified T-cell according the invention for the preparation of a medicament.

The invention also provides a method for the treatment of an individual suffering from or at risk of suffering from a disease associated with at least part of the immune system of said individual comprising providing said individual with an dendritic cell and/or a functionally modified T-cell according to the invention. Preferably, said dendritic cell and/or said functionally modified T-cell, or precursors thereof are derived from an HLA-matched donor. Preferably, said HLA-matched donor is said individual.

Method of treatments of the invention are preferably use for the treatment of an individual suffering from an auto-immune disease, an allergy, a graft versus host disease and/or a host versus graft disease.

Examples

Example 1

Impairment of CD40-CD40L-mediated phenotypic changes by DEX

We explored the impact of DEX on the phenotypic changes induced by CD40 ligation on immature monocyte-derived DC. In the absence of DEX, the fusion protein CD8-CD40L induced a strong upregulation of the costimulatory molecules CD80, CD86 and CD40, of the MHC class I and II molecules, of the adhesion markers CD54 and CD58 and of the DC maturation marker CD83 (Fig 1). In the presence of DEX, these CD8-CD40L-induced phenotypic changes were dramatically impaired: the upregulation of CD80, CD86, CD40, CD54, CD58 and of the MHC class I and II molecules was largely inhibited and CD83 was not expressed (Fig 1). Importantly, DEX-treated DC did not revert to a monocyte/macrophage stage as shown by the lack of expression of CD14 (Fig 1). Titration of DEX showed a complete inhibition of CD40-mediated phenotypic changes at 10^{-6} M and 10^{-7} M, a partial blockade at 10^{-8} M and no effect at 10^{-9} M and 10^{-10} (data not shown). In addition, DEX action was dependent on binding to the GC-receptor, since it was asbolished by simultaneous addition of the GC receptor antagonist RU486 (data not shown). In experiments performed with LPS or TNF-a as activation agents, similar results were obtained. However, the combination of DEX and TNF-alpha induced a massive cell death (viable cell recovery 5-10% of control cultures), a phenomenon that was not observed when DEX-treated DC were stimulated with LPS or through CD40 (viable cell recovery 60 to 100% of control cultures) (not shown).

We next analyzed whether activated DC could still be affected by DEX. DC incubated with CD8-CD40L for 48h and further exposed to DEX maintained a stable activated phenotype (Fig 2).

We conclude that DEX prevents the phenotypic changes induced by CD40 signals on immature DC and that already activated DC are resistant to DEX action.

Example 2

DEX does not interfere with the regulation of DC antigen uptake machinery

Unlike activated DC, immature DC efficiently internalize antigens through macropinocytosis and mannose receptor-mediated endocytosis (2, 3, 25, 26). We analyzed whether DEX could affect the DC antigen capture machinery and its downregulation following CD40 cross-linking. As shown in Fig 3, incorporation of FITC-BSA and FITC-mannosylated BSA by immature DC and by DEX-treated immature DC was comparable. Upon CD40 triggering, a similar decrease of FITC-BSA and FITC-mannosylated BSA uptake by both DEX-treated and untreated DC was observed (Fig 3). These results were the first to indicate to us that DEX does not block all aspects of DC activation, since it does not interfere with the down-regulation of the DC antigen capture machinery.

Example 3

DEX-treated CD40-triggered DC secrete IL-10 instead of IL-12

A key feature of CD40-triggered DC for initiating T cell immunity resides in their ability to produce the proinflammatory cytokine IL-12 (5, 6, 27). We investigated whether DEX affected IL-12 production by DC stimulated through CD40, and we explored the possibility that DEX could promote the secretion of the anti-inflammatory cytokine IL-10. As shown in Fig 4, CD40 triggering of DC strongly induced IL-12p40 and IL-12p70 secretion (up to 120ng/ml and 170pg/ml respectively) but only poorly stimulated the production of IL-10 (up to 68pg/ml). In contrast, CD40 triggering of DEX-treated DC resulted in a dramatically reduced IL-12p40 production (up to 100 fold) and in the complete suppression of IL-12p70 secretion, whereas IL-10 production was strongly

enhanced (up to 50 fold) (Fig 4). Immature DC and their DEX-treated counterparts failed to secrete detectable amounts of IL-12 and IL-10 (Fig 4). Therefore, CD40 ligation of DC in the presence of DEX triggers the secretion of high levels of the anti-inflammatory cytokine IL-10 instead of IL-12.

Example 4

DEX-treated CD40-triggered DC are capable of suppressing Th1-type immunity

The strikingly modified response of DC to CD40 ligation in the presence of DEX prompted us to compare the T cell stimulatory potential of these cells with that of their DEX-untreated counterparts. In an allogeneic MLR, CD40-triggered DC induced a strong proliferative T cell response whereas the addition of DEX prior to CD40 triggering reduced their T cell stimulatory capacity to that of immature DC (Fig 5). When tested for their ability to stimulate an hsp65-specific $CD4^+$ Th1 clone, CD40-triggered DC pulsed with the hsp65 protein or with the specific peptide epitope p3-13 were found to be potent inducers of both T cell proliferation and T-cell dependent IFN-g production (Fig 5). In contrast, in the presence of Ag-pulsed DEX-treated CD40-triggered DC, T cell proliferation and IFN-g production were significantly decreased ($p<0.001$ and $p<0.01$ respectively) (Fig 5). We next investigated whether DEX-treated CD40-triggered DC were simply poor stimulators of Th1 cells, or whether they could exert suppressive effects on these T cells. We therefore tested hsp65-specific T cells stimulated with p3-13-pulsed DEX-treated CD40-triggered DC for their capacity to respond to a second potent antigenic challenge. Fig 6 shows that preculturing T cells with CD40-triggered DC led to a strong T cell proliferation and IFN-gamma production upon second antigen-specific restimulation. In contrast, preculture with DEX-treated CD40-triggered DC resulted in a dramatically reduced proliferative and IFN-gamma production capacity of Th1 cells. Thus, CD40 triggering of DC in the presence of DEX

results in APC that are not merely poor inducers of T cell responses but that also induce a state of hyporesponsiveness in Th1 cells.

Materials and Methods

Generation of DC

Immature DC were generated from peripheral blood monocyte precursors. Human PBMC from healthy donors, isolated through Ficoll-Hypaque density centrifugation were plated at $1.5x10^7$ per well in 6-well plates (Costar Corp., Cambridge, MA) in RPMI 1640 (Life Technologies, Paisley, Scotland) supplemented with 2mM glutamine, 100UI/ml penicillin and 10% FCS. After 2 h at 37^0C, the non adherent cells were removed and the adherent cells were cultured in medium containing 500U/ml IL-4 (Pepro Tech Inc. Rocky Hill, NJ) and 800U/ml GM-CSF (kindly provided by Dr S. Osanto, LUMC, Leiden, NL) for a total of 7 days.

Activation of immature DC with a CD8-CD40L fusion protein

Activation of DC though CD40 was performed with a fusion protein made of the extracellular domain of human CD40L and of the murine CD8a chain (CD8-CD40L). The CD8-CD40L cDNA described by Garrone et al (23) was transferred into an eukaryotic expression vector containing the hygromycin resistance gene, and used for the generation of stably transfected Chinese Hamster Ovary (CHO) cells. Culture supernatants containing the CD8-CD40L fusion protein were concentrated with a pressurized stirred cell system (Amicon, Inc., Beverly, MA), checked for binding to CD40 and tested for optimal DC activation conditions (not shown). DC were incubated at 5×10^5/ml/well in a 24-well plate (Costar Corp., Cambridge, MA) and activated in the presence of 1/10 CD8-CD40L supernatant. Cells and supernatants were analyzed after 48 h. Of note, control supernatants obtained from

untransfected CHO cells or from CHO cells transfected with the CD8a cDNA lacked DC activating functions and were similar to culture medium.

DEX and RU486 treatment of DC

Seven days immature DC were treated with 10^{-6}M DEX (Sigma, St Louis, MO) in the presence of GM-CSF and IL-4 or GM-CSF alone. After 24 h, DC were analyzed or were further stimulated via CD40 by adding the CD8-CD40L fusion protein to the cultures as described above. In some experiments, the glucocorticoid receptor antagonist RU485 (Roussel-UCLAF, Romainville, France) was used at 10mM final concentration, alone or in combination with DEX.

Analysis of DC surface phenotype by flow cyttometry

Cells were stained on ice with FITC or PE-conjugated mouse monoclonal antibodies (MoAb) for 30 min in PBS 1% FCS and were analyzed on a FACScan® (Becton Dickinson, San Jose, CA). The following MoAb were used: FITC-anti-CD80 (BB1), PE-anti-CD86 (FUN-1), FITC-anti-CD40 (5C3), PE-anti-CD54 (HA 58) and PE-anti-CD58 (1C3) (Pharmingen, San Diego, CA), PE-anti-CD14 (L243) and PE-anti-HLA-DR (Mf-P9) (Becton Dickinson), PE-anti-CD83 (HB15A) (Immunotech, Marseille, France) and PE-anti-HLA class I (Tu 149) (Caltag Laboratories, Burlingame, CA).

Antigen uptake experiments

DC were resuspended in medium buffered with 25mM Hepes. FITC-BSA and FITC-mannosylated BSA (both from Sigma) were added at 1mg/ml final concentration and the cells were incubated at 37^0C, or at 0^0C to determine background uptake. After 1 h, DC were washed extensively with iced-cold PBS and analyzed by FACS® using propidium iodide to eliminate dead cells.

Cytokine detection by ELISA

Culture supernatants were analyzed in serial twofold dilutions in duplicate. IL-12p70 was detected using a solid phase sandwich ELISA kit (Diaclone Research, Besancon, France) (sensitivity 3pg/ml). For IL-12p40 and IFN-g detection, capture MoAb and polyclonal biotinylated detection Ab were obtained from Peter van de Meijde (BPRC, Rijswijk, NL) (sensitivity 10pg/ml). IL-10 was detected using the Pelikine compact human IL-10 ELISA kit (CLB, Amsterdam, NL) (sensitivity 3pg/ml).

Allogeneic mixed lymphocyte reaction (MLR)

Non adherent allogeneic adult PBMC from an unrelated individual were cultured in 96-well flat-bottom plates (Costar Corp., Cambridge, MA) at a density of 1.5×10^5/well with various numbers of g-irradiated (3,000 rads) DC, in triplicates. Proliferation was assessed on day 5 by [^{3}H]thymidine uptake (0.5mCi/well, specific activity 5Ci/mMol, Amersham Life Science, Buckinghamshire, UK) during a 16 h pulse.

Th1 stimulation assays

The Mycobacterium tuberculosis and M. leprae hsp65-specific, HLA-DR3-restricted CD4+ Th1 clone Rp15 1-1 used in this study recognizes an hsp65 determinant corresponding to peptide residues 3 to 13 (p3-13) (24). HLA-DR-matched DEX-treated immature DC and their DEX-untreated counterparts were pulsed with 10mg/ml of p3-13 or with 10mg/ml of hsp65 for 2 h, washed extensively and stimulated through CD40 as described above. For Ag-pulsed DEX-treated immature DC, CD40 triggering was performed in the presence of DEX. Hsp65 specific T cells (10^4) were cultured with different numbers of g-irradiated (3,000 rads) DC in 96-well flat-bottom plates (Costar Corp.) in triplicates for 3 days. [^{3}H]thymidine incorporation was measured on day 3 after a 16 h pulse. Before the addition of

[^{3}H]thymidine, 50ml of supernatants were collected from each well and supernatants from triplicate wells were pooled to measure IFN-g production. To test hsp65-specific T cells responsiveness to a second potent antigenic challenge, 10^4 T cells were first cultured for 48h with 5 x 10^3 peptide-pulsed DC prepared as above, then harvested and allowed to rest in medium containing 5U/ml IL-2. Three days later, 10^4 viable T cells were restimulated with 5 x 10^3 peptide-pulsed DC generated from the same donor as used for the first culture and tested for their ability to proliferate and to produce IFN-g as previously described.

Statistical analysis

Covariance analysis was used to compare T cell proliferation and IFN-g production as a function of DC number, between DEX-treated CD40-triggered DC and DEX-untreated CD40-triggered DC (Fig. 5).

Figure legends

Fig. 1 Pretreatment with DEX inhibits the phenotypic changes induced by CD40 ligation.

Seven days immature DC were cultured for 24h in the absence or the presence of 10^{-6}M DEX and activated via CD40 with the CD8-CD40L fusion protein for 48h. The comparison with immature DC maintained in medium alone is shown. Empty histograms show the background staining with isotype controls MoAb and solid histograms represent specific staining of the indicated cell surface markers. Specific mean fluorescence intensities are indicated. Mean fluorescence intensities of isotype controls were between 3 and 4. Data are representative of 4 independent experiments.

Fig. 2 DC triggered through CD40 maintain an activated phenotype upon a subsequent DEX exposure.

Immature DC were activated with the CD8-CD40L fusion protein. DEX (10^{-6}M) or medium control were added 48h later and cells were analyzed after 2 additional days of culture. The comparison with immature DC maintained in medium alone is shown. Empty histograms show the background staining with isotype controls MoAb and solid histograms represent specific staining of the indicated cell surface markers. Specific mean fluorescence intensities are indicated. Mean fluorescence intensities of isotype controls were between 3 and 5. Data are representative of 2 independent experiments.

Fig. 3 Pretreatment with DEX does not affect the regulation of DC antigen uptake machinery.

Immature DC were incubated in the absence or the presence of 10^{-6}M DEX for 24h and further activated or not via CD40 with the CD8-CD40L fusion protein for 48h. Cells were pulsed for 1h with medium containing either 1mg/ml FITC-BSA or 1mg/ml

FITC-mannosylated BSA. Empty histograms show the background autofluorescence, Grey-filled histograms show the background uptake at 0^0C and black-filled histograms show the specific uptake at 37^0C. Data are representative of 3 independent experiments.

Fig. 4 Pretreatment with DEX alters the cytokine secretion profile of CD40-triggered DC.
DEX-exposed or control immature DC were left in culture without further treatment or stimulated with the CD8-CD40L fusion protein. Culture supernatants were harvested 48h later and IL-10, IL-12p40 and IL-12p70 secretion were analyzed by specific ELISA. Data are representative from 6 independent experiments.

Fig. 5 Pretreatment with DEX impairs the T cell stimulatory capacities of DC activated via CD40 and leads to a state of hyporesponsiveness of Th1 cells.
Allogeneic MLR: non adherent allogeneic PBMC were cultured with different numbers of CD40-triggered DC, DEX-treated CD40-triggered DC or immature DC. The proliferative response was measured on day 5.
Th1 stimulation assays: Hsp65-specific T cells were cultured with different numbers of HLA-DR matched CD40-triggered DC or with DEX-treated CD40-triggered DC pulsed with the hsp65 protein or with the specific p3-13 peptide epitope. The proliferative response and the T cell dependent IFN-g production were analyzed on day 3. Data are representative of 4 independent experiments.

Fig. 6 DEX-treated DC triggered through CD40 induce a state of hyporesponsiveness in Th1 cells. Hsp65-specific T cells precultured with CD40-triggered DC or with DEX-treated CD40-triggered DC pulsed with the p3-13 peptide epitope were harvested after 48h, allowed to rest in the presence of 5U/ml

IL2 for 3 days, and restimulated with p3-13-pulsed DC. The proliferative response and IFN-g production were measured on day 3. Similar results were obtained in 2 independent experiments.

REFERENCES

1. Banchereau J, Steinman RM: Dendritic cells and the control of immunity. Nature 392: 248, 1998

2. Sallusto F, Lanzavecchia A: Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony stimulating factor and downregulated by tumor necrosis factor a. J Exp Med 179: 1109, 1994

3. Sallusto F, Cella M, Danieli C, Lanzavecchia A: Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. J Exp Med 182: 389, 1995

4. Caux C, Massacrier C, Vandervliet B, Dubois B, van Kooten C, Durand I, Banchereau J: Activation of human dendritic cells through CD40 cross-linking. J Exp Med 180: 1263, 1994

5. Cella M, Sheidegger D, Palmer-Lehman K, Lane P, Lanzavechia A, Alber G: Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T-T help via APC activation. J Exp Med 184: 747, 1996

6. Koch F, Stanzl U, Jennevin P, Janke K, Heufler C, Kampgen E, Romani N, Schuler G: High levels IL-12 production by murine dendritic cells: upregulation via MHC class II and CD40 molecules and downregulation by IL-4 and IL-10. J Exp Med 184: 741, 1996

7. Trinchieri G: Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. Annu Rev Immunol 13: 251, 1995

8. Kamanaka M, Yu P, Yasui T, Koshida K, Kawaba T, Horii T, Kishimoto T, Kikutani H: Protective role of CD40 in Leishmania major infection at two distinct phases of cell-mediated immunity. Immunity 4: 275, 1996

9. Yang Y, Wilson JM: CD40 ligand-dependent T cell activation: requirement of B7-CD28 signaling through CD40. Science 273: 1862, 1996

10. Mackey MF, Gunn JR, Ting P, Kikutani H, Dranoff G, Noelle RJ, Barth Jr RJ: Protective immunity induced by tumor vaccines requires interactions between CD40 and its ligand CD154, Cancer Res 57: 2569, 1997

11. Grewal IS, Foellmer HG, Grewal KD, Xu J, Hardardottir F, Baron JL, Janeway Jr CA, Flavell RA: Requirement for CD40 ligand in costimulation induction, T cell activation and experimental allergic encephalomyelitis. Science 273: 1864, 1996

12. Kirk AD, Harlan DM, Armstrong NN, Davis TA, Dong Y, Gray GS, Hong X, Thomas D, Fechner JH, Knechtle SJ: CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates. Proc Natl Acad Sci USA 94: 8789, 1997

13. Durie FH, Aruffo A, Ledbetter J, Crassi KM, Green WR, Fast LD, Noelle RJ: Antibody to the ligand of CD40, gp39, blocks the occurrence of the acute and chronic forms of graft-vs-host disease. J Clin Invest 94: 1333, 1994

14. Almawi WY, Beyhum HN, Rahme AA, Rieder MJ: Regulation of cytokine and cytokine receptor expression by glucocorticoids. J Leukoc Biol 60: 563, 1996

15. Moser M, De Smedt T, Sornasse T, Tielemans F, and Chentoufi AA: Glucocorticoids down-regulate dendritic cell function in vitro and in vivo. Eur J Immunol 25: 2818, 1995

16. Kitajima T, Ariitzumi K, Bergstresser PR, Takashima A: A novel mechanism of glucocorticoid-induced immune suppression: the inhibition of T cell-mediated terminal maturation of a murine dendritic cell line. J Clin Invest 98: 142, 1996

17. Viera PL, Kalinski P, Wierenga EA, Kapsenberg ML, de Jong EC: Glucocorticoids inhibit bioactive IL-12p70 production by in vitro-generated human dendritic cells without affecting their T cell stimulatory potential. J Immunol 161: 5245, 1998

18. Romani N, Gruner S, Brang D, Kampgen E, Lenz A, Trockenbacher B, Konwalinka G, Fritsch PO, Steinman RM,

Schuler G: Proliferating dendritic cell progenitors in human blood. J Exp Med 180: 83, 1994

19. Randolph GC, Beaulieu S, Lebecque S, Steinman RM, Muller WA: Differentiation of monocytes into dendritic cells in a model of transendothelial trafficking. Science 282: 480, 1998

20. Rissoan MC, Soumelis V, Kadowaki N, Grouard G, Briere F, de Waal Malefyt R, Liu Y-J: Reciprocal control of T helper cell and dendritic cell differentiation. Science 283: 1183, 1999

21. Bender A, Sapp M, Schuler G, Steinman RM, Bhardwaj N: Improved methods for the generation of dendritic cells from non proliferating progenitors in human blood. J Immunol Methods 196:121, 1996

22. Romani N, Reider D, Heuer M, Ebner S, Kampgen E, Eibl B, Niederwieser D, Schuler G: Generation of mature dendritic cells from human blood: an improved method with special regard to clinical applicability. J Immunol Methods 196:137, 1996

23. Garrone P, Neidhardt E-M, Garcia E, Galibert L, van Kooten C, Banchereau J: Fas ligation induces apoptosis of CD40-activated human B lymphocytes. J Exp Med 182: 1265, 1995

24. Geluk A, van Meigaarden KE, Janson AAM, Drijfhout J-W, Meloen R, de Vries RRP, Ottenhoff T: Functional analysis of DR17(DR3)-restricted mycobacterial T cell epitopes reveals DR17 binding motif and enables the design of allele-specific competitor peptides. J Immunol 149: 2864, 1992

25. Engering AJ, Cella M, Fluitsma D, Brockhaus M, Hoefsmit ECM, Lanzavecchia A, Pieters J: The mannose receptor functions as a high capacity and broad specificity antigen receptor in human dendritic cells. Eur J Immunol 27: 2412, 1997

26. Tan MCAA, Mommaas AA, Drijfhout J-W, Jordens R, Onderwater JJM, Verwoerd D, Mulder AA, van der Heiden AN, Scheidegger D, Oomens LCJM, Ottenhoff THM, Tulp A, Neefjes JJ, Koning F: Mannose receptor-mediated uptake of antigens strongly enhances HLA class II-restricted antigen

presentation by cultured dendritic cells. Eur J Immunol 27: 2426, 1997

27. de Saint Vis B, Fugier-Vivier I, Massacrier C, Gaillard C, Vanbervliet B, Ait-Yahia S, Banchereau J, Liu Y-J, Lebecque S, Caux C: The cytokine profile expressed by human dendritic cells is dependent on cell subtype and mode of activation. J Immunol 160: 1666, 1998

28. Piemonti L, Monti P, Allavena P, Sironi M, Soldini L, Leone BE, Socci C, Di Carlo V: Glucocorticoids affect human dendritic cell differentiation and maturation. J Immunol 162: 6473, 1999

29. Blotta MH, Dekruyff RH, Umetsu DT: Corticosteroids inhibit IL-12 production in human monocytes and enhance their capacity to induce IL-4 synthesis in CD4+ lymphocytes. J Immunol 158: 5589, 1997

30. Visser J, van Boxel-Dezaire A, Methrost D, Brunt T, de Kloet ER, Nagelkerken L: Differential regulation of interleukin 10 (IL-10) and IL-12 by glucocorticoids in vitro. Blood. 91: 4255, 1998

31. van Kooten C, Banchereau J: Function of CD40 on B cells, dendritic cells and other cells. Curr Opin Immunol 9: 330, 1997

32. Scheinman RI, P.C. Cogswell PC, Lofquist AK, and Baldwin Jr AS: Role of transcriptional activation of I kappa B alpha in mediation of immunosuppression by glucocorticoids. Science 270: 283, 1995

33. Auphan N, Dinato JA, Rosette C, Helmberg A, Karin N: Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. Science 270: 286, 1995

34. Murphy TL, Cleveland MG, Kulesza P, Magram J, Murphy KM: Regulation of interleukin 12 p40 expression through an NF-kappa B half site. Mol Cell Biol 15: 5258, 1995

35. Yoshimoto T, Nagase H, Ishida T, Inoue J, Nariuchi H: Induction of interleukin-12 p40 transcript by CD40 ligation

via activation of nuclear factor-kappaB. Eur J Immunol 27: 3461, 1997

36. Ridge JP, Di Rosa F, Matzinger P: A conditioned dendritic cell can be a temporal bridge between a CD4+ T-helper and a T-killer cell. Nature 393: 474, 1998

37. Bennett SR, Carbone FR, Karamalis F, Flavell RA, Miller JF, Heath WR: Help for cytotoxic-T-cell responses is mediated by CD40 signalling. Nature 393: 478, 1998

38. Schoenberger SP, Toes REM, van der Voort EI, Offringa R, Melief CJM: T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. Nature 393: 480, 1998

CLAIMS

1. A method for preparing a pharmaceutical composition for reducing an unwanted T cell response in a host, comprising culturing peripheral blood monocytes from said host to differentiate into dendritic cells, activating said dendritic cells in the presence of a glucocorticoid hormone and loading said dendritic cells with an antigen against which said T cell response is to be reduced.

2. A pharmaceutical composition for reducing an unwanted T cell response in a host, said composition being obtained by culturing peripheral blood monocytes from said host to differentiate into dendritic cells, activating said dendritic cells in the presence of a glucocorticoid hormone and loading said dendritic cells with an antigen against which said T cell response is to be reduced.

3. A method for reducing an unwanted T cell response in a host, comprising administering a composition of claim 2 to said host.

4. A method for reducing an unwanted T cell response in a host comprising culturing peripheral blood monocytes from said host to differentiate into dendritic cells, activating said dendritic cells in the presence of a glucocorticoid hormone and loading said dendritic cells with an antigen against which said T cell response is to be reduced and administering said composition to said host.

5. A method according to claim 1,3 or 4 whereby said activation is done through a CD40 receptor.

6. A method according to claim 5 whereby said activation involves incubation of the dendritic cells with either CD8-CD40L fusion protein, a trimeric from of CD40L consisting of CD40L-molecules to which a modified leucine zipper has been attached, anti-CD40 antibodies, or cells that express CD40L.

7. A method according to claim 5 whereby said activation involves incubation of the dendritic cells with lipopolysaccharide (LPS) or polyI/C.

8. A method according to claim 1, 3-7 whereby said dendritic cells are infected with one or more recombinant viruses encoding the antigen(s) of interest before activating said dendritic cells in the presence of a glucocorticoid hormone.

9. A method according to claim 1, 3-8 whereby said dendritic cells are incubated with one or more recombinant proteins or large (> 20 amino acids) synthetic peptides representing the antigen(s) of interest before activating said dendritic cells in the presence of a glucocorticoid hormone.

10. A method according to claim 1,3 or 9 whereby said dendritic cells are incubated with cells or cell homogenate containing the antigen(s) of interest before activating said dendritic cells in the presence of a glucocorticoid hormone.

11. A method according to claim 1, 3-10 whereby said dendritic cells are loaded with synthetic peptides representing the antigen(s) of interest after activating said dendritic cells in the presence of a glucocorticoid hormone.

12. A method according to claim 1, 3-11 whereby said dendritic cells, after activation in the presence of a glucocorticoid hormone, secrete interleukin-10.

13. A method for obtaining a dendritic cell capable of tolerising a T-cell for an antigen comprising providing said dendritic cell with a glucocorticoid hormone, activating said dendritic cell and providing said dendritic cell with said antigen.

14. A method according to anyone of claims 1, 3-13, wherein said dendritic cell and/or a precursor thereof is provided with said glucocorticoid hormone in vitro.

15. A method according to anyone of claims 1, 3-14, wherein said T-cell is a T-helper cell.

16. An isolated dendritic cell prepared according to anyone of claims 1, 3-15 capable of functionally modifying an antigen-specific T-cell with respect to the response to said antigen.

17. A method for functionally modifying an antigen-specific T-cell comprising providing an dendritic cell according to claim 16 with said antigen and co-cultivating said T-cell and said dendritic cell.

18. A method according to claim 17, wherein said co-cultivating is performed in vitro.

19. A method according to claim 17 or claim 18, further comprising multiplying said functionally modified T-cell.

20. An isolated functionally modified T-cell obtainable by a method according to anyone of claims 17-19 that is capable, upon administration to the host, of reducing an unwanted immune response.

21. Use of a glucocorticoid hormone for obtaining an dendritic cell capable of functionally modifying a T-cell.

22. A pharmaceutical composition comprising an dendritic cell according to claim 16 and/or a functionally modified T-cell according to claim 20.

23. Use of an dendritic cell according to claim 16 and/or a functionally modified T-cell according to claim 20 for the preparation of a medicament.

24. A method for the treatment of an individual suffering from or at risk of suffering from a disease associated with at least part of the immune system of said individual comprising providing said individual with an dendritic cell according to claim 16 and/or a functionally modified T-cell according to claim 20.

25. A method according to claim 24, wherein said dendritic cell and or said T-cell is derived from an HLA-matched donor.

26. A method according to claim 24 or claim 25, wherein said dendritic cell and or said T-cell is derived from said individual.

27. Use of an dendritic cell according to claim 16 in a treatment for an individual suffering from an auto-immune disease, allergy, a graft versus host disease and/or a host versus graft disease.

Title: Dendritic cell activated in the presence of glucocorticoid hormones are capable of suppressing antigen-specific T cell responses.

ABSTRACT

The present invention provides novel methods for immunotherapy. The invention provides immune cells and methods to generate them, with the capacity to at least in part reduce an immune response in a host. In one aspect the invention provides a method for generating a dendritic cell with the capacity to tolerise a T cell for antigen said T cell was specific for, comprising culturing peripheral blood monocytes from an individual to differentiate into dendritic cells, activating said dendritic cells in the presence of a glucocorticoid hormone and loading said activated dendritic cell with said antigen said T cell was specific for.

Fig. 1

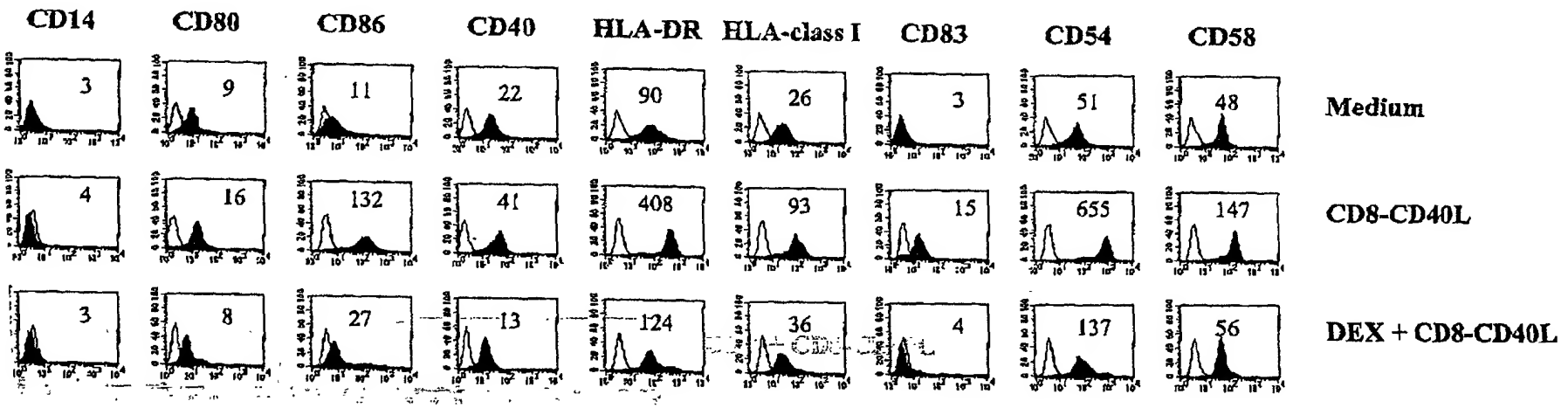

CD14
CD80
CD86
CD40
HLA-DR
HLA-class I
CD83
CD54
CD58
3
9
11
22
90
26
3
51
48
Medium
4
16
132
41
408
93
15
655
147
CD8-CD40L
3
8
27
13
124
36
4
137
56
DEX + CD8-CD40L

Fig. 2

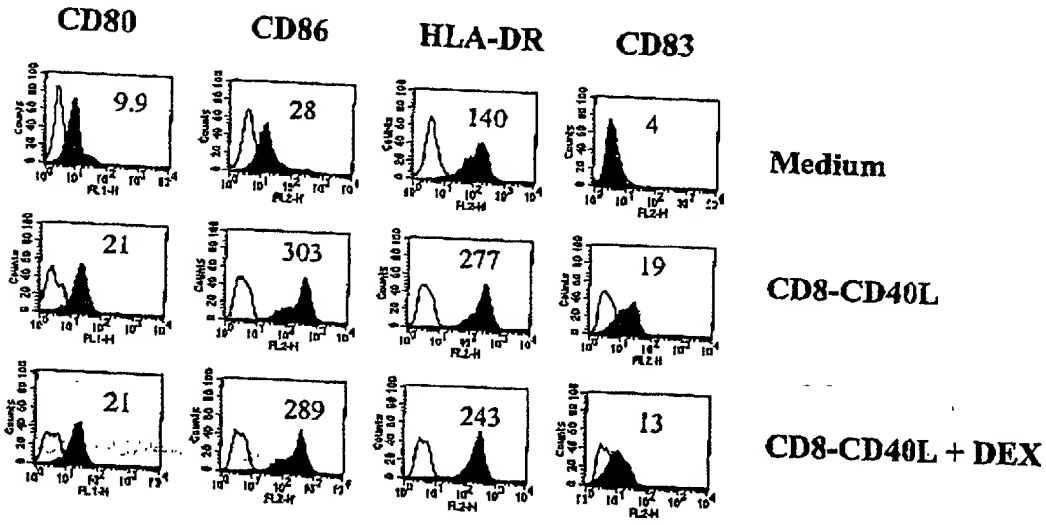

CD80
CD86
HLA-DR
CD83
9.9
28
140
4
21
303
277
19
21
289
243
13
Medium
CD8-CD40L
CD8-CD40L + DEX

Fig. 3

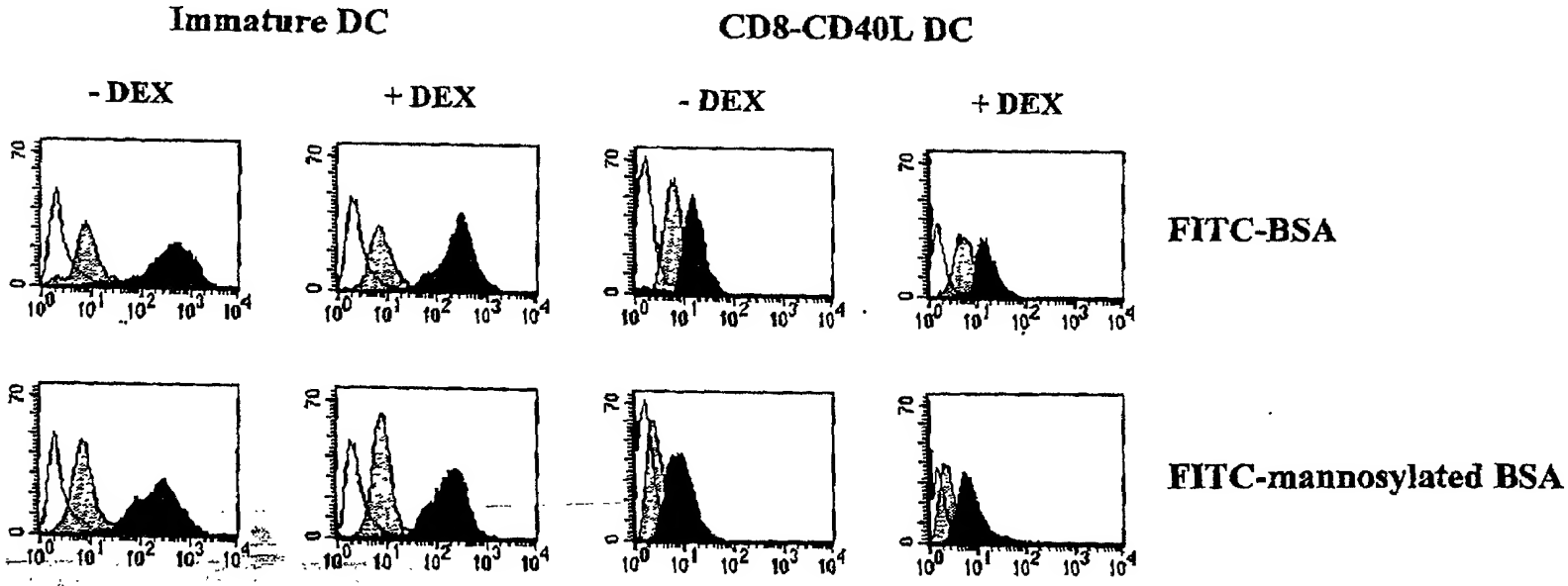
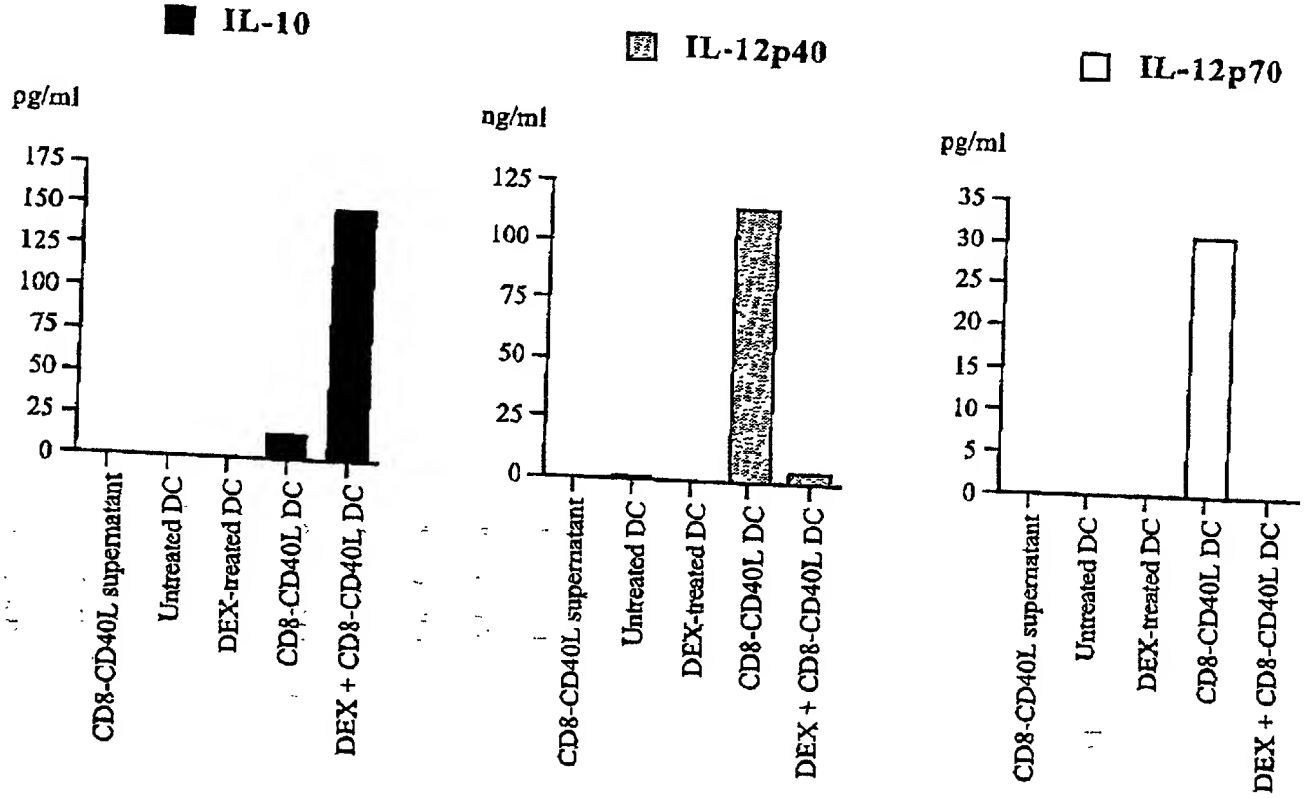

Immature DC
CD8-CD40L DC
- DEX
+ DEX
- DEX
+ DEX
FITC-BSA
FITC-mannosylated BSA

Fig. 4



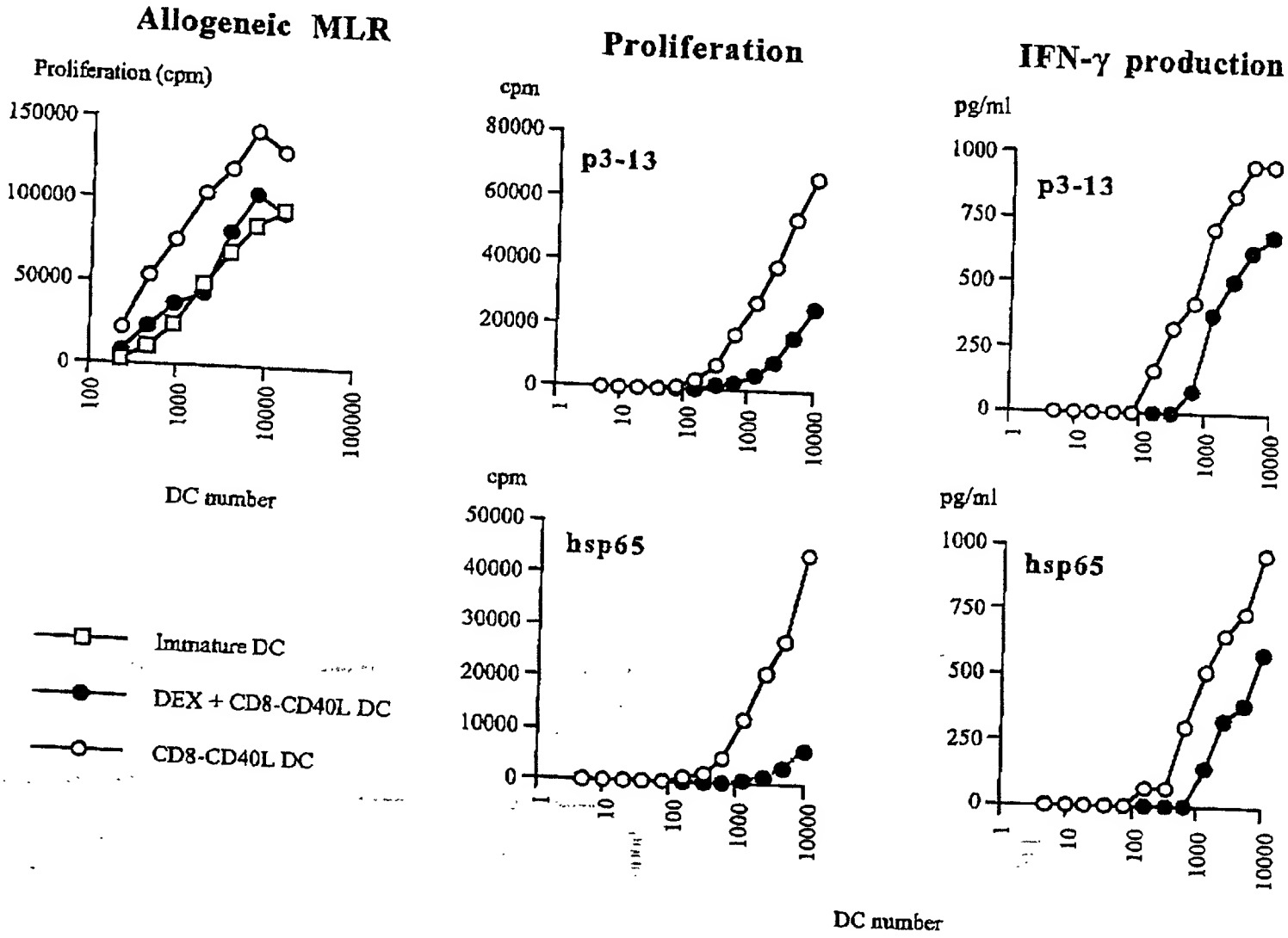
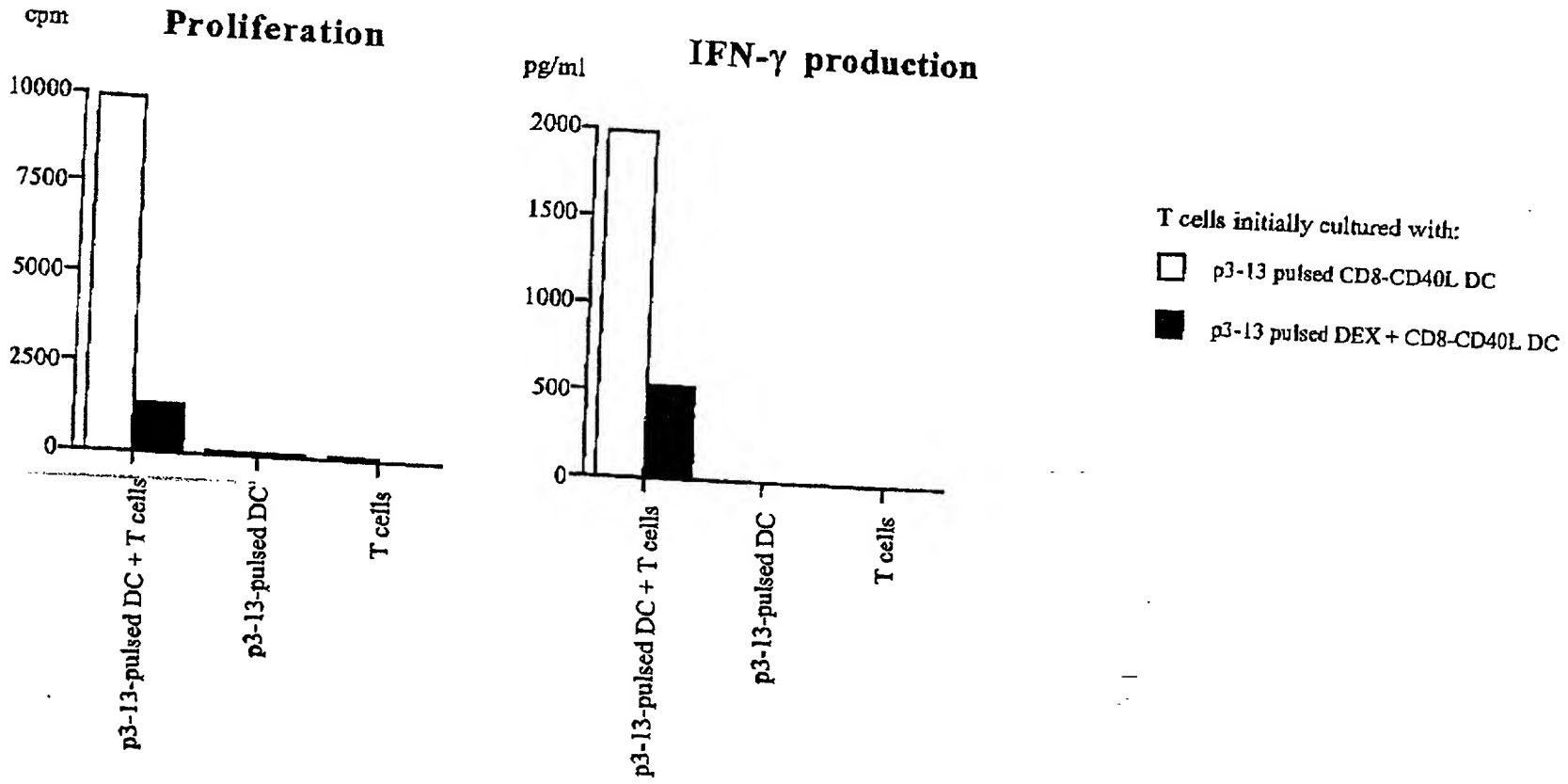


Fig. 5

Fig. 6



Attorney Docket No. 4205.1US

DECLARATION FOR PATENT APPLICATION (WITH POWER OF ATTORNEY)

As an inventor named below or on any attached continuation page, I hereby declare that:

My residence, post office address and citizenship are as stated next to my name.

I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled DENDRITIC CELL ACTIVATED IN THE PRESENCE OF GLUCOCORTICOID HORMONES ARE CAPABLE OF SUPPRESSING ANTIGEN-SPECIFIC T CELL RESPONSES, the specification of which (check one):

- ☒ is attached hereto.
- ☐ was filed on _____ as United States application serial no. _____ and was amended on _____.
- ☐ was filed on _____ as PCT international application no. _____ and was amended under PCT Article 19 on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to the patentability of the subject matter claimed in this application, as "materiality" is defined in Title 37, Code of Federal Regulations § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate or § 365(a) of any PCT international application(s) designating at least one country other than the United States of America listed below and on any attached continuation page and have also identified below and on any attached continuation page any foreign application for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America having a filing date before that of the application(s) on which priority is claimed.

Prior foreign/PCT application(s):

			Priority Claimed	
(number)	(country)	(day/month/year filed)	Yes	No
(number)	(country)	(day/month/year filed)	Yes	No

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or § 365(c) of PCT international application(s) designating the United States of America listed below and on any attached continuation page and, insofar as the subject matter of each of the claims of this application is not disclosed in any such prior application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which became available between the filing date of such prior application and the national or PCT international filing date of this application:

(application serial no.)	(filing date)	(status - pending, patented or abandoned)
(application serial no.)	(filing date)	(status - pending, patented or abandoned)

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

60/157,442	October 4, 1999
(provisional application no.)	(filing date)

I hereby appoint the following Registered Practitioners to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

David V. Trask, Reg. No. 22,012
Laurence B. Bond, Reg. No. 30,549
Allen C. Turner, Reg. No. 33,041
Stephen R. Christian, Reg. No. 32,687
Paul C. Oestreich, Reg. No. 44,983
Eleanor V. Goodall, Reg. No. 35,162
Kerry D. Tweet, Reg. No. P-45,959
William S. Britt, Reg. No. 20,969
Joseph A. Walkowski, Reg. No. 28,765
Kent S. Burningham, Reg. No. 30,453
Brick G. Power, Reg. No. 38,581
Devin R. Jensen, Reg. No. 44,805
Kenneth C. Booth, Reg. No. 42,342
Thomas J. Rossa, Reg. No. 26,799
James R. Duzan, Reg. No. 28,393
Edgar R. Cataxinos, Reg. No. 39,931
Kenneth B. Ludwig, Reg. No. 42,814
David L. Stott, Reg. No. 43,937
Samuel E. Webb, Reg. No. 44,394

Address all correspondence to: Allen C. Turner, telephone no. (801) 532-1922.
TRASK, BRITT & ROSSA
P.O. BOX 2550
Salt Lake City, Utah 84110

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of first joint inventor: Delphine Gabrielle Josette Rea
Inventor's signature _____ Date April 4 2000
Residence: Leiden, The Netherlands
Citizenship: Dutch
Post Office Address: Floresstraat 1, 2315 HP Leiden, The Netherlands

Attorney Docket No. 4205.1US

DECLARATION FOR PATENT APPLICATION
(continuation page)

Invention title: DENDRITIC CELL ACTIVATED IN THE PRESENCE OF GLUCOCORTICOID HORMONES ARE CAPABLE OF SUPPRESSING ANTIGEN-SPECIFIC T CELL RESPONSES

Inventor name(s) appearing on first declaration page: Delphine Gabrielle Josette Rea

☒ Additional original, first and joint inventor(s):

Full name of second joint inventor: Cornelis Johannes Maria Melief
Inventor's signature _____ Date April 4, 2000
Residence: Haarlem, The Netherlands
Citizenship: Dutch
Post Office Address: Wilhelminapark 33, 2012 KC Haarlem, The Netherlands

Full name of third joint inventor: Rienk Offringa
Inventor's signature _____ Date April 3, 2000
Residence: Leiden, The Netherlands
Citizenship: Dutch
Post Office Address: Stieltjesstraat 63, 2313 SJ Leiden, The Netherlands